Association of Photosystem I and Light-Harvesting Complex II during State Transitions

Boekema, Egbert J.; Kouřil, Roman; Dekker, Jan P.; Jensen, Poul Erik

Published in:
Photosystem I: The Light-Driven Plastocyanin: Ferredoxin Oxidoreductase.

Publication date:
2006

Chapter 5

Association of Photosystem I and Light-Harvesting Complex II during State Transitions

Egbert J. Boekema* and Roman Kouřil
Department of Biophysical Chemistry, GBB, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Jan P. Dekker
Division of Physics and Astronomy, Faculty of Sciences, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

Poul Erik Jensen
Plant Biochemistry Laboratory, Department of Plant Biology, The Royal Veterinary and Agricultural University, 40 Thorvaldsenvej, Frederiksberg C DK-1871, Denmark

Summary

Green plant photosystem I (PS I) not only binds a chlorophyll *a*/*b*-binding, membrane-intrinsic antenna complex (LHCI) that is associated with the PS I core complex under almost all physiological conditions, but it can also transiently bind the major chlorophyll *a*/*b*-binding light-harvesting complex (LHCII), when the light conditions favor excitation of photosystem II (PS II) and the photosynthetic apparatus is in the so-called state 2. Recently, a low-resolution structure was obtained of a PS I–LHCII supercomplex from *Arabidopsis thaliana*. We describe here some of the structural features of this transient complex, and discuss the role of small PS I subunits that are involved in the binding of LHCII. We also discuss structural features of the PS I complex of the green algae *Chlamydomonas reinhardtii*, which has a larger LHCI antenna and shows a more pronounced difference between state 1 and state 2.

I. Introduction

One of the ways by which plants adapt to changing light conditions is to change the direction of part of the absorbed light to either PS I or PS II. The response of the photosynthetic apparatus to such light fluctuations is called a state transition (Allen and Forsberg,

* Author for correspondence, email: e.j.boekema@rug.nl

Abbreviations: EM – electron microscopy; LHCI – light-harvesting complex I; LHCII – light-harvesting complex II; PS I – photosystem I; PS II – photosystem II; PS I-A to PS I-O – alternative higher plant nomenclature for PS I polypeptides.

John H. Golbeck (ed): Photosystem I: The Light-Driven Plastocyanin:Ferredoxin Oxidoreductase, 41–46.

2001; Haldrup et al., 2001; Wollman, 2001). State transitions occur in three main steps. The first step is the initiation of a signal transduction leading to the activation of kinases. This activation is brought about by the reduced state of the plastoquinone (PQ) pool and the cytochrome b_6f complex, induced by preferential excitation of PS II (Vener et al., 1997; Zito et al., 1999). The second step is the phosphorylation, by the activated kinases, of light-harvesting complex II (LHCII). In plants, the major LHCII proteins consist of three closely related chlorophyll *a*/*b*-binding proteins encoded by the *Lhcb1–3* genes (Jansson, 1994; see also Croce et al., this volume, Chapter 10). Two kinase families are proposed to be involved in LHCII phosphorylation: TAK kinases (Snyders and Kohorn, 1999, 2001) and STT7 kinases in *Chlamydomonas reinhardtii* (Depège et al., 2003) and its homolog in *Arabidopsis* STN7 (Bellafiore et al., 2005). Mutants without those kinases cannot perform phosphorylation of LHCII and have impaired state transitions. The phosphorylation of LHCII is thought to cause a conformational change, which allows LHCII to diffuse from the grana to PS I in the stromal parts of the thylakoid membrane, constituting the last step in the state transition (Allen and Forsberg, 2001). The binding of phosphorylated LHCII to PS I brings the system to state 2 and allows a redistribution of the light energy between the two photosystems and a balancing of linear electron flow. This association between PS I and LHCII is reversible. Preferential excitation of PS I leads to oxidation of the plastoquinol pool, a dephosphorylation of LHCII, and a release of LHCII from the PS I–LHCII complex (state 1).

The three main steps in the state transitions are rather similar in green plants and green algae such as *C. reinhardtii*, but in some respects these systems are very different. The extent of the state transition is much larger in *C. reinhardtii*, in which 80% of LHCII may migrate between PS II and PS I (Delosme et al., 1996), whereas in plants only 10–20% of LHCII migrates between PS II and PS I (Allen, 1992). The differences in the extent of the state transitions may relate to the amount of stacking, which is much lower in *C. reinhardtii*. A less tight stacking may result in a greater accessibility of LHCII for the kinases and a larger proportion of LHCII that can migrate to PS I (Dekker and Boekema, 2005). In *C. reinhardtii*, the transition from state 1 to state 2 is accompanied by an increase of cyclic electron transfer around PS I (Finazzi et al., 2002), whereas in *Arabidopsis thaliana*, no change in cyclic electron transfer occurs upon state transitions (Lunde et al., 2003). Furthermore, the structure of the PS I–LHCI complex is different in both types of organisms. LHCI of green plants consists of four chlorophyll *a*/*b*-binding proteins, encoded by the *Lhca1–4* genes (Jansson, 1994), while LHCI of *C. reinhardtii* consists of nine proteins of the Cab gene family (Takahashi et al., 2004). The structure of the PS I–LHCI complex from pea is known at 4.4 Å resolution (Ben-Shem et al., 2003), and shows that the four LHCI proteins are located in a row at the PS I-FJ side of the complex between the PS I-G and PS I-K subunits. A low-resolution structure of the PS I–LHCI complex from *C. reinhardtii* was obtained by single-particle electron microscopy (Germano et al., 2002; Kargul et al., 2003). It was suggested that four of the additional LHCI proteins are bound in a second row flanked by the PS I-G and PS I-K proteins, and that a single LHCI protein is bound at the other side of the complex between PS I-H, PS I-A, and PS I-K (Dekker and Boekema, 2005).

Despite intensive search, no structural information on the putative PS I–LHCII complex in state 2 was available until very recently. On the basis of both cross-linking experiments and the structures of the green plant PS I–LHCI and LHCII complexes, Ben-Shem and Nelson proposed a location of LHCII at the PS I-A side between PS I-H and PS I-K (Ben-Shem and Nelson, 2005). Recently, we obtained a structure of the top view of a PS I–LHCII complex from *Arabidopsis* in state 2 (Kouřil et al., 2005), in which the LHCII is slightly displaced compared to the suggestion of Ben-Shem and Nelson. The new structure gives new information on the interaction between PS I–LHCI and LHCII, and forms the main topic of this chapter.

II. Structure of the PS I–LHCII Complex

Thylakoid membranes of *A. thaliana* were prepared in state 1 or state 2 by red or orange light treatment, respectively, as described by Zhang and Scheller (2004). The membranes were solubilized with the very mild detergent digitonin, and immediately without further purification analyzed by electron microscopy (Kouřil et al., 2005). This procedure prevents disassembly of the particles as much as possible, and allows a statistical analysis of the numbers of the various complexes in the membranes. Figure 1 shows a typical electron micrograph of the solubilized material. Many of the projections can be recognized because of their particular size and shape, such as ATPase, $C_2S_2M_2$ PS II–LHCII supercomplexes (Yakushevska et al., 2001), and PS I–LHCI particles (Boekema et al., 2001). All projections from particles with a size similar or larger than that of the PS I–LHCI complex (see also Nelson and

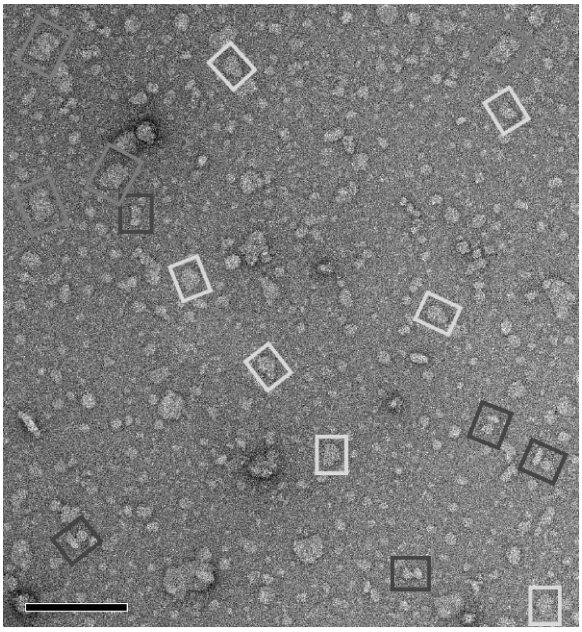

Fig. 1. Part of an electron micrograph of nonpurified single particles from digitonin solubilized thylakoid membranes of *Arabidopsis thaliana* in state 2. Projections of PSI/PSI–LHCII, PSII, and ATPases are indicated by white, gray, and black boxes, respectively. The scale bar equals 100 nm. (Modified from Kouřil et al., 2005.)

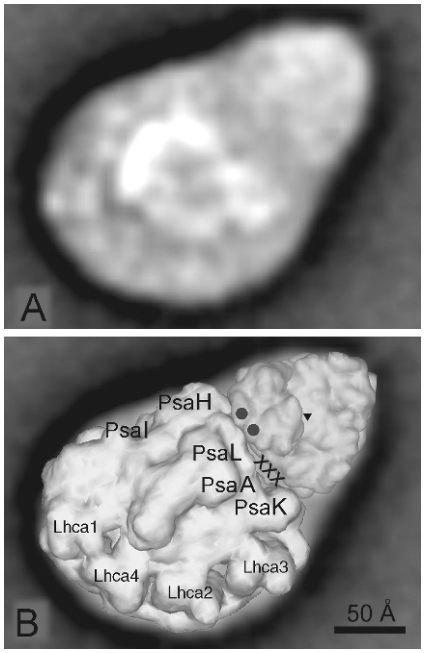

Fig. 2. Model of the PSI–LHCII supercomplex. (A) Final projection map at 16 Å. (B) See Color Plate 1, Fig. 1.

Ben-Shem, this volume, Chapter 7) were selected and subjected to multivariate statistical analysis. The results showed that the majority of the analyzed PS I projections from state 2 membranes belong to classes of oval-shaped PS I–LHCI particles, or to novel pear-shaped particles (Fig. 2A), representing a supercomplex of PS I and trimeric LHCII (see below). The same projections were obtained from state 1 membranes, but with the pear-shaped particles in much smaller numbers.

The results obtained by Kouřil et al. (2005) have a number of implications. One implication is that at least the majority of LHCII that binds to PS I is trimeric. More than 90% of the analyzed PS I particles could be attributed to either the normal PS I–LHCI complex, or to the same complex with associated trimeric LHCII, which excludes the occurrence of significant amounts of supercomplexes of PS I and monomeric LHCII. In older literature (see, e.g., Bassi et al., 1988) it was suggested that the migrating LHCII could be monomeric, but in these papers the "state 2" was obtained by high light, which is not an appropriate way to induce the state transition. High light can, for instance, induce monomerization of LHCII (Garab et al., 2002), so the earlier proposed involvement of monomeric LHCII may be the result of the applied illumination protocol. It was also suggested that phosphorylation induces conformational changes of LHCII, one of which is a trimer-to-monomer transition (Nilsson et al., 1997). Our results show that a trimer-to-monomer transition is not required for binding to PS I and that the amount of bound monomers in state 2 is very small.

Another implication is that the binding of PS I and LHCII is specific. If the binding of LHCII to PS I was not specific, the final image (Fig. 2A) would have been more blurred, or there would have been more than one class of projections with differences in the LHCII area. A final fitting of the atomic PS I and LHCII structures into the electron microscopy projection map (Fig. 2B) clearly resembles the proposal of Ben-Shem and Nelson (2005) based on the fits of the intermediate-resolution structures of PS I and LHCII, though the LHCII in our image is slightly displaced toward the PS I-K subunit.

III. Role of Small PS I Subunits in State Transitions

Earlier work has shown that plants without PS I-H and PS I-L (Lunde et al., 2000) and without PS I-O (Jensen et al., 2004) are highly deficient in state transitions (Zhang and Scheller, 2004). In addition, cross-links could be established between LHCII and PS I-I, PS I-H,

and/or PS I-L. The new PS I–LHCII structure shows that the LHCII trimer is attached at a defined position between subunits PS I-A, -H, -L, and -K (Fig. 2B). The involvement of PS I-H and PS I-L in the binding of LHCII is expected in view of the role of these subunits in the state transitions and of the detected cross-link to LHCII. The involvement of PS I-K in the binding of LHCII is however surprising, because the absence of PS I-K by the addition of an antisense construct does not impair state transitions to any great extent (Jensen et al., 2000) and a cross-link between PS I-K and LHCII was not detected (Zhang and Scheller, 2004). The absence of PS I-K even resulted in a slightly enhanced binding of LHCII (Zhang and Scheller, 2004), which can be explained by a small shift of LHCII toward PS I-A. This is possible because in the presence of PS I-K there seems to be a small gap between PS I-A and LHCII (indicated as XXX in Fig. 2B). It is not known where the PS I-O subunit is located. This protein was not found in the PS I–LHCI crystal structure, but without this protein the state transitions are 50% impaired (Jensen et al., 2004). A cross-link with LHCII was not found, but particles isolated from mutants without PS I-O have smaller amounts of attached LHCII (Zhang and Scheller, 2004), which indicates that this protein is at least indirectly involved in the binding of LHCII. Recently the discovery of a new subunit of PS I was reported (Khrouchtchova et al., 2005). This subunit, which is denoted PS I-P, appears to be associated with PS I-L in the proximity of PS I-O and -H and it is likely that PS I-P also is involved in the interaction with LHCII. The exact role of these small membrane-intrinsic subunits in state transitions need further functional characterization.

Figure 2B also shows that the observed cross-link between PS I-I and LHCII (Zhang and Scheller, 2004) is hard to explain by the structure of the current PS I–LHCII complex. It is possible though that there is a second binding site of LHCII, which is even weaker than the first and therefore can be missed in our analysis. Cross-linking will prevent the detachment of the cross-linked complex, so in this type of analysis such a binding might be revealed. If a second binding site exists, it may be present at the symmetry-related position covered by the PS I-H, -I, -B, and -G subunits. Further work is needed to clarify this point.

IV. Origin of LHCII Bound to PS I

The results of Kouřil et al. (2005) show that solubilization of state 2 membranes results in slightly lower numbers of PS II–LHCII supercomplexes with four bound LHCII complexes and somewhat higher numbers of supercomplexes with three bound LHCII complexes. This suggests that among the LHCII complexes that move from the grana to PS I there are at least some that originate from the so-called M-LHCII, the LHCII that is bound to the PS II core dimer at the site of the CP29 and CP24 units (Dekker and Boekema, 2005). However, it is hard to make quantitative statements on the origin of the LHCII that migrates to PS I. It is possible that some LHCII arises from supercomplexes that were completely disintegrated during the state transition, because the remaining complexes are too small to be picked up and analyzed by our EM analysis. It is also possible that a considerable part of the migrating LHCII originates from LHCII-only parts of the grana (Boekema et al., 2000).

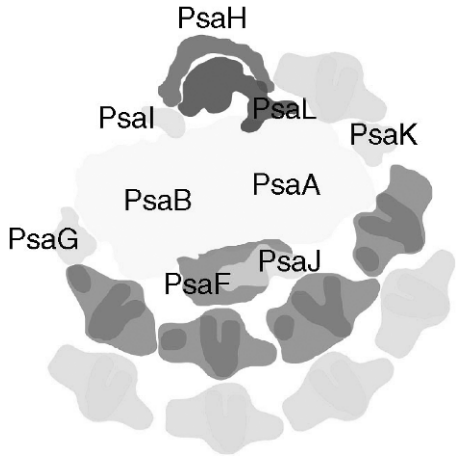

Fig. 3. Model of the PSI–LHCI complex of *Chlamydomonas reinhardtii* with an inner row of four peripheral LHCI proteins (darker shade), and with four LHCI proteins in a second row (lighter shade) plus one single LHCI protein (lighter shade) at the position of the LHCII trimer in green plants. (Modified from Dekker and Boekema, 2005.)

V. State Transitions in *C. reinhardtii*

It is now well-established that the PS I–LHCI complex from the green alga *C. reinhardtii* is larger than that of green plants (Germano et al., 2002; Kargul et al., 2003). In this organism, PS I binds nine monomeric LHCI subunits (Takahashi et al., 2004), eight of which are most likely bound at the PS I-FJ side of the complex in two rows between the PS I-G and PS I-K (Fig. 3), and one at the other side of the complex between PS I-H and PS I-K. This is the same position at which

trimeric LHCII binds in state 2 in *A. thaliana* (see above). An analysis of *n*-dodecyl-β-D-maltoside solubilized PS I–LHCII particles from *C. reinhardtii* prepared in state 1 or state 2 revealed no differences in the positions of the nine LHCI proteins (A. E. Yakushevska, unpublished observations). This suggests that the binding site of LHCII on green plant PS I is always occupied by a monomeric LHC type of protein in PS I of *C. reinhardtii*. This also suggests significant differences in the binding of LHCII to PS I in both types of organisms.

However, there may be a relation between a relatively small mass at the tip of the complex, near the PS I-H protein, and state transitions. Multivariate statistical analysis of single-particle projections from PS I–LHCI particles prepared in a state between a pure state 1 and a pure state 2 (Germano et al., 2002) revealed two types of complexes with a small size difference near PS I-H. The difference is too small for the binding of one additional trimeric LHCII complex in the larger complex, but fits more or less that of a monomeric LHCII-type of complex. Preliminary results indicate that the smaller complex is more abundant in state 1 (in agreement with Kargul et al., 2003), and that the larger complex is more abundant in state 2. However, the additional mass in the larger particle is much too small to explain the large extent of the state transition in *C. reinhardtii*. It is in this respect interesting to note that PS II–LHCII super- and megacomplexes could only be isolated from *C. reinhardtii* membranes grown in state 1, but not in state 2 (A. E. Yakushevska, unpublished observations), suggesting that the association of PS II and LHCII is less tight in state 2 than in state 1. So, the disintegration of majority of the PS II–LHCII supercomplexes could form a major contributor to the state transition in this organism. Digitonin solubilization and an immediate analysis of all solubilized complexes, as done for *Arabidopsis* (Kouřil et al., 2005) should provide more information on the possible presence of a PS I–LHCII complex in *C. reinhardtii* in state 2.

Acknowledgment

This work was supported by the European Union, Grant HPRN-CT-2002-00248 (PSICO network).

References

Allen JF (1992) Protein phosphorylation in regulation of photosynthesis. Biochim Biophys Acta 1098: 275–335

Allen JF and Forsberg J (2001) Molecular recognition in thylakoid structure and function. Trends Plant Sci 6: 317–326

Bassi R, Giacometti GM and Simpson DJ (1988) Changes in the organization of stroma membranes induced by in vivo state-1–state-2-transition. Biochim Biophys Acta 935: 152–165

Bellafiore S, Barneche F, Peltier G and Rochaix JD (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature 433: 892–895

Ben-Shem A and Nelson N (2005) System biology of photosystem I – formation of supercomplexes. In: Van der Est A and Bruce D (eds) Photosynthesis: Fundamental Aspects to Global Perspectives, pp 770–772. Alliance Communications Group, Lawrence, Kansas

Ben-Shem A, Frolow F and Nelson N (2003) Crystal structure of plant photosystem I. Nature 426: 630–635

Boekema EJ, van Breemen JFL, van Roon H and Dekker JP (2000) Arrangement of photosystem II supercomplexes in crystalline macrodomains within the thylakoid membrane of green plant chloroplasts. J Mol Biol 301: 1123–1133

Boekema EJ, Jensen PE, Schlodder E, van Breemen JFL, van Roon H, Scheller HV and Dekker JP (2001) Green plant photosystem I binds light-harvesting complex I on the side of the complex. Biochemistry 40: 1029–1036

Dekker JP and Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim Biophys Acta 1706: 12–39

Delosme R, Olive J and Wollman F-A (1996) Changes in light energy distribution upon state transitions: an *in vivo* photoacoustic study of the wild type and photosynthetic mutants from *Chlamydomonas reinhardtii*. Biochim Biophys Acta 1273: 150–158

Depège N, Bellafiore S and Rochaix JD (2003) Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. Science 299: 1572–1575

Finazzi G, Rappaport F, Furia A, Fleischmann M, Rochaix J-D, Zito F and Forti G (2002) Involvement of state transitions in the switch between linear and cyclic electron flow in *Chlamydomonas reinhardtii*. EMBO Rep 3: 280–285

Garab G, Cseh Z, Kovacs L, Rajagopal S, Varkonyi Z, Wentworth M, Mustardy L, Der A, Ruban AV, Papp E, Holzenburg A and Horton P (2002) Light-induced trimer to monomer transition in the main light-harvesting antenna complex of plants: thermo-optic mechanism. Biochemistry 41: 15141–15149

Germano M, Yakushevska AE, Keegstra W, van Gorkom HJ, Dekker JP and Boekema EJ (2002) Supramolecular organization of photosystem I and light-harvesting complex I in *Chlamydomonas reinhardtii*. FEBS Lett 525: 121–125

Haldrup A, Jensen PE, Lunde C and Scheller HV (2001) Balance of power: a view of the mechanism of photosynthetic state transitions. Trends Plant Sci 6: 301–305

Jansson S (1994) The light-harvesting chlorophyll *a*/*b* proteins. Biochim Biophys Acta 1184: 1–19

Jensen PE, Gilpin M, Knoetzel J and Scheller HV (2000) The PSI-K subunit of photosystem I is involved in the interaction between light-harvesting complex I and the photosystem I reaction center core. J Biol Chem 275: 24701–24708

Jensen PE, Haldrup A, Zhang S and Scheller HV (2004) The PSI-O subunit of plant photosystem I is involved in balancing the excitation pressure between the two photosystems. J Biol Chem 279: 24212–24217

Kargul J, Nield J and Barber J (2003) Three-dimensional reconstruction of a light-harvesting complex I-photosystem

I (LHCI-PSI) supercomplex from the green alga *Chlamydomonas reinhardtii* – insights into light harvesting for PSI. J Biol Chem 278: 16135–16141

Khrouchtchova A, Hansson M, Paakkarinen V, Vainonen JP, Zhang S, Jensen PE, Scheller HV, Vener AV, Aro E-M and Haldrup A (2005) A previously found thylakoid membrane protein of 14 kDa (TMP14) is a novel subunit of plant photosystem I and is designated PSI-P. FEBS Lett 29: 4808–4812

Kouřil R, Zygadlo A, Arteni AA, de Wit CD, Dekker JP, Jensen PE, Scheller HV and Boekema EJ (2005) Structural characterization of a complex of photosystem I and light-harvesting complex II in *Arabidopsis thaliana*. Biochemistry 44: 10935–10940

Lunde C, Jensen PE, Haldrup A, Knoetzel J and Scheller HV (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature 408: 613–615

Lunde C, Jensen PE, Rosgaard L, Haldrup A, Gilpin MJ and Scheller HV (2003) Plants impaired in state transitions can to a large degree compensate for their defect. Plant Cell Physiol 44: 44–54

Nilsson A, Stys D, Drakenberg T, Spangfort MD, Forsén S and Allen JF (1997) Phosphorylation controls the three-dimensional structure of plant light-harvesting complex II. J Biol Chem 272: 18350–18357

Snyders S and Kohorn BD (1999) TAKs, thylakoid membrane protein kinases associated with energy transduction. J Biol Chem 274: 9137–9140

Snyders S and Kohorn BD (2001) Disruption of thylakoid-associated kinase 1 leads to alteration of light harvesting in Arabidopsis. J Biol Chem 276: 32169–32176

Takahashi Y, Yasui T-A, Stauber EJ and Hippler M (2004) Comparison of the subunit compositions of the PSI–LHCI supercomplex and the LHCI in the green alga *Chlamydomonas reinhardtii*. Biochemistry 43: 7816–7823

Vener AV, van Kan PJ, Rich PR, Ohad I and Andersson B (1997) Plastoquinol at the quinol oxidation site of reduced cytochrome bf mediates signal transduction between light and protein phosphorylation: thylakoid protein kinase deactivation by a single-turnover flash. Proc Natl Acad Sci USA 94: 1585–1590

Wollman FA (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J 20: 3623–3630

Yakushevska AE, Jensen PE, Keegstra W, van Roon H, Scheller HV, Boekema EJ and Dekker JP (2001) Supermolecular organization of photosystem II and its associated light-harvesting antenna in *Arabidopsis thaliana*. Eur J Biochem 268: 6020–6028

Zhang S and Scheller HV (2004) Light-harvesting complex II binds to several small subunits of photosystem I. J Biol Chem 279: 3180–3187

Zito F, Finazzi G, Delosme R, Nitschke W, Picot D and Wollman FA (1999) The Qo site of cytochrome $b(6)f$ complexes controls the activation of the LHCII kinase. EMBO J 8: 2961–2969